

THE ANATOMY OF THE BARKS OF FIVE SPECIES OF *CALLITRIS* VENT.

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(Plates xx-xxi.)

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Synopsis.

In this paper, detailed anatomical descriptions of the bark of five species of *Callitris* Vent. are given. A key for the identification of these five species based on the bark anatomy has been devised.

The resin canals are considered to be schizogenous rather than lysigenous in origin as recorded elsewhere. The presence of bordered pits and unusual thickening of phloem fibre walls is noted. Crystal sand of unknown constitution has been found in the middle lamella of the radial walls of the cells. The stringy and compact types of bark found in *Callitris* have been related to the anatomy.

INTRODUCTION.

The Australian genus *Callitris* and the ornamental species *Cupressus macrocarpa* Hartweg differ markedly in their resistance to the cypress pine jewel beetle. While *Callitris* spp. are able to survive attacks by this beetle on the phloem, cambium and outer sapwood, *Cupressus macrocarpa* readily succumbs. To assist in the study of the cypress pine jewel beetle by Hadlington and Gardner (1959), this study of the anatomy of five species of *Callitris* and comparison with the anatomy of *Cupressus macrocarpa* and *C. lusitanica* has been made.

A broad study of the bark anatomy of the genus *Callitris* Vent. was included by Baker and Smith (1910) in their research on the pines of Australia, but details of the cell dimensions, the nature of the expansion of the phloem parenchyma and the differences in structure between various species were not given. Their account of resin canal formation and the terms used for the description of the bark anatomy are also in need of revision in the light of descriptions by the more recent authors on bark anatomy (Eames and McDaniels, 1947; Esau, 1950 and 1953; Chang, 1952; and Chattaway, 1954).

MATERIALS.

The following species were selected for study: *Callitris hugelii* (Carr) Franco (Syn. *C. glauca* R.Br.), 4 trees; *Callitris endlicheri* (Parl) Syn. *C. calcarata* (A. Cunn. ex Mirb) F. Muell., 4 trees; *Callitris macleayana* F. Muell., 3 trees; *Callitris rhomboidea* R.Br. ex A. et L. C. Rich., 4 trees; *Callitris intratropica* Baker and Smith, 3 trees; *Cupressus macrocarpa* Hartweg, 3 trees (planted); *Cupressus lusitanica* Miller, 2 trees (planted).

The nomenclature for the *Callitris* is taken from the revision of the genus by Garden (1957).

METHODS.

The material was prepared for examination by means of standard techniques using Johansen (1940) as a guide. It was found more satisfactory to use isopropyl alcohol for dehydration prior to embedding.

For the observation of crystal sand it was found necessary to remove tanniniferous materials by treatment with acetic acid and hydrogen peroxide. This solution also served for maceration. Softening of bark with chemical treatment prior to sectioning was not necessary.

In the calculation of volumes of fibres, the linear method of Chalk (1956) was used. For the calculation of volume of resin canals the method described by Brown,

Panshin and Forsaith (1949) was followed, in which photomicrographs are weighed before and after the various tissues are cut out. This method was found to be more rapid and accurate than a planimeter.

DESCRIPTION OF BARK ANATOMY.

General.

While the barks of all species of *Callitris* examined are made up of phloem, narrow periderm and a persistent rhytidome built of successive layers of dead phloem separated by fine periderm layers, the general appearance of the barks and the thickness of barks vary considerably. Both *C. hugelii* and *C. endlicheri* develop, in mature trees, a thick outer bark (up to $1\frac{1}{2}$ in.) with large vertical furrows, the texture of the bark being hard and compact. On the other hand *C. rhomboidea* develops a hard compact outer bark which is much thinner, up to $\frac{1}{4}$ in., and has small vertical furrows. Both *C. intratropica* and *C. macleayana* develop a loose fibrous outer bark. In the former this outer bark is somewhat thinner (up to $\frac{1}{2}$ in.) and more compact than in the latter (up to $1\frac{1}{2}$ in.) which is well known as the stringybark cypress, having a very loose fibrous bark.

The inner bark (phloem) of all species is thin, approximately $\frac{1}{8}$ in., and very hard. When the bark is freshly cut the inner phloem appears cream to yellow, turning reddish towards the periderm and darkening to brown-black in the outer bark.

The resin canals are clearly seen with the eye, the resin exuding freely from the canals of the phloem. In the rhytidome the resin is solid, the canals appearing as white dots.

Arrangement of Phloem Tissues.

The phloem of all species of *Callitris* examined is made up of regular concentric layers of sieve cells, phloem fibres and phloem parenchyma together with phloem rays. The layers are repeated in the following sequence: sieve cell, fibre, sieve cell, parenchyma. This sequence is consistently maintained in all species examined and does not appear to be seasonal.

Phloem Fibres.

The phloem fibres of all species of *Callitris* examined appeared thick-walled with small lumen, thickening of the wall occurring rapidly after division from the cambium and being completed three or four cells from the cambium.

The walls of the fibres are profusely pitted (Pl. xx, fig. 1), the pits being bordered, the chamber very small and the aperture narrowed axially (Pl. xx, fig. 2). Due to the profuse pitting and large degree of thickening of the walls, the lumen surface of the fibre shows a characteristic corrugated appearance in the longitudinal direction (Pl. xx, fig. 1). Also unusual is the tendency for the pit aperture to be oriented diagonally. While many pits are oriented towards the radial walls forming pit pairs, some are oriented towards the tangential wall or else diagonally without forming a pit pair and are thus blind.

Phloem fibres of *Callitris* are characteristically rectangular in section, being considerably wider, tangentially (15 to 45μ), than radially (9 to 27μ).

Some differences between species is apparent in the average fibre length. In *C. endlicheri*, *C. hugelii* and *C. rhomboidea* it is approximately the same, being 2.6 mm., 2.7 mm. and 2.6 mm., respectively; in *C. intratropica* it is 3.2 mm. and in *C. macleayana* 3.6 mm. These differences are paralleled somewhat by the differences in the average fibre length of the xylem, for example, *C. hugelii* 2.4 mm., *C. endlicheri* 3.1 mm., *C. rhomboidea* 2.6 mm., *C. macleayana* 4.2 mm. and *C. intratropica* 4.2 mm.

The volume of phloem occupied by fibres ranges from 18% in *C. endlicheri* to 26% in *C. macleayana*. In the outer bark (rhytidome) these percentages do not hold, due to expansion of the parenchyma.

Sieve Cells.

Sieve cells are the principal cells of *Callitris* phloem and comprise 50% of the phloem cells, apart from the rays. They appear generally similar in size to the phloem fibres, measuring from 15 to 27 μ tangentially and from 9 to 27 μ radially. The length of the sieve cells has not been measured, but they appear to be a little shorter than the fibres.

The rectangular appearance of the fibres in transverse section is not always shared by the sieve cells. In the inner phloem they are inclined to be swollen, compressing the adjacent parenchyma cells, whereas in the outer phloem the sieve cells become in turn compressed by the expanding parenchyma cells. In *Callitris macleayana* the sieve cells retain their rounded form in the outer phloem and rhytidome and it is the adjacent parenchyma which is collapsed (Pl. xx, fig. 3). Sieve areas are clearly defined, being more or less circular and about 15 μ in diameter. The sieve areas are profuse and are spaced at from 3 to 10 μ apart. The sieve pits are clearly defined in the older sieve cells (Pl. xxi, fig. 10).

The ends of the sieve cells are rounded. The wall of the sieve cell is thin, not lignified in the inner phloem, and with no secondary thickening.

Phloem Parenchyma.

The parenchyma cells of the inner phloem are much shorter than the adjacent sieve cells and fibres, being from 0.06 to 0.12 mm. long; but they are of similar tangential and radial dimensions, being 15–27 μ by 9–27 μ .

Towards the outside of the phloem and in the inner rhytidome, significant changes occur in the phloem parenchyma, due to the expansion of these cells and change in the nature and amount of the tanniniferous deposits. In *Callitris hugelii* the phloem parenchyma in the outer phloem measured up to 60 μ tangentially by 115 μ radially, with *C. endlicheri*, 27 μ by 60 μ (Pl. xx, fig. 4), and *C. rhomboidea*, 75 μ by 115 μ . In *C. macleayana*, no significant change in the size of the phloem parenchyma cells is observed. However, after the outer phloem cells are cut off from the inner phloem by the formation of a deeper seated periderm, expansion of occasional concentric layers of phloem parenchyma occurs (Pl. xx, fig. 3), the parenchyma cells in other layers collapsing. Only a small amount of expansion occurs in the phloem parenchyma in the phloem and rhytidome of *C. intratropica* (Pl. xxi, fig. 5).

In *C. endlicheri*, *C. hugelii* and *C. rhomboidea*, the expansion of the phloem parenchyma partially destroys the regular concentric formation of phloem cells (Pl. xx, fig. 4, and Pl. xxi, fig. 6). In *C. macleayana* and *C. intratropica* the regular concentric pattern is maintained in the fibrous layers of the rhytidome (Pl. xx, fig. 3).

The tannin-like deposits of the phloem parenchyma cells appeared granular and pale brown in the phloem. After separation from the living phloem by the periderm, the tannin-like material becomes dark red-brown in colour and separates as a solid material from the cell walls. These deposits are very prolific in *C. endlicheri*, *hugelii*, *intratropica* (Pl. xxi, fig. 5) and *rhomboidea*, but scarce in *C. macleayana*.

Phloem Rays.

Phloem rays are uniseriate and mostly four or five cells high. In the inner phloem, the deposits of tanniniferous materials are pale in colour, but towards the outer phloem they become much darker and more abundant. Some small enlargement of phloem ray cells occurs, particularly in the region of division of phloem parenchyma or formation of resin canal. The tannin-like materials of the ray parenchyma change in colour and appearance between the phloem and rhytidome as with the phloem parenchyma.

Resin Canals.

The resin canals are formed by division and subsequent separation of parenchyma cells, the epithelial cells of the canal being thin-walled (Pl. xxi, fig. 9). This division commences very close to the cambium. After formation, the resin canals increase in size until they become part of the rhytidome. The division of parenchyma to form

canals occurs in *C. endlicheri* in about every 4th to 6th row of parenchyma and follows a concentric pattern. The canals continue vertically for a considerable distance and in *C. macleayana* were measured to 4 in. Resin canal formation appears to be of natural occurrence in the bark of *Callitris* spp., their frequency being a function of species rather than injury. There is considerable variation, in both size and frequency of resin canals of the phloem, in the five species examined, as shown in the following table:

Species.	Number of Resin Canals per sq. mm.	Size of Resin Canals.
<i>Callitris endlicheri</i>	14 to 20	0.08 to 0.16 mm.
<i>C. hugelii</i>	6 to 13	0.13 to 0.19 mm.
<i>C. intratropica</i>	1.3 to 3.8	0.21 mm.
<i>C. rhomboidea</i>	1.3	0.09 to 0.4 mm.
<i>C. macleayana</i>	0.5 to 0.75	0.21 mm.

Periderm.

Periderm formation occurs by division of phloem parenchyma; it is concentric in pattern and is similar in all the species of *Callitris* examined. The phelloderm is from two to three cells wide, while the phellogen consists of a single or double layer of thin-walled cells. The phellem produced varies from 3 to 6 cells in width. The phellem cells are thin-walled and free of deposits, whereas the phelloderm frequently shows intense tanniferous deposits. The phellem cells measure approximately 0.03 to 0.054 mm. in height.

In the rhytidome of a tree approximately 20 years old, 10 corky layers were counted. The corky layers (phellem) appear as fine pale-brown bands more or less concentric.

Rhytidome.

The rhytidome of *Callitris* consists mainly of old phloem tissue which has been cut off by successive periderms and fine cork layers. As was shown in the observations of phloem parenchyma, the various forms of the rhytidome are due to the characteristics of these parenchyma cells.

Nature of Cell Walls.

When stained with safranin and fast green the secondary wall of the phloem fibres shows lignification close to the region of differentiation. The primary wall of the phloem fibres does not show lignification until the outer phloem or rhytidome does, the thin primary wall readily separating from the secondary wall on sectioning. The primary walls of the other cells of the phloem behave similarly to this staining reaction, lignification not being found until the outer phloem or rhytidome is lignified.

The primary walls of all the phloem cells give a positive reaction for cellulose with iodine and sulphuric acid.

Crystal Sand.

Abundant deposits of a fine crystalline material were found in *C. hugelii*, *C. endlicheri*, *C. rhomboidea*, *C. intratropica*, *Cupressus macrocarpa* and *Cupressus lusitanica*. The crystal sand is deposited in the middle lamella of the radial walls of all cells, and is laid down at the time of fibre differentiation. It appears to extend through the phloem in radial rows within the middle lamella. These deposits were not found in *C. macleayana*. This material appears similar to the crystal sand reported by Chang (1952) as occurring in the sieve cells of *Chamaecyparis thyoides* (L.) B.S.P. The crystal sand is difficult to distinguish in a normal preparation, due to the large amounts of tanniferous material present, and treatment with acetic acid and hydrogen peroxide is required to remove the deposits before observation can be made. It can be observed, however, in thin sections particularly if polarized light is used. The crystals are insoluble in a mixture of 30 per cent. hydrogen peroxide and glacial acetic acid, strong sulphuric acid, hydrofluoric acid, sodium hydroxide and ammonium hydroxide. The chemical nature is unknown.

DISCUSSION.

The characteristic form of *Callitris* bark in which uniseriate bands or layers of sieve cells alternate with uniseriate layers of phloem fibres and phloem parenchyma was first shown by Baker and Smith (1910). Mention of this alternating concentric phloem structure has been made for the members of the family Cupressaceae by De Bary (1877, reported by Takamatsu, 1928), and Takamatsu (1928), and for *Sequoia sempervirens* Endl. and *Libocedrus decurrens* Torrey by Abbe and Crafts (1939). Chang (1952) makes use of the alternating structure as a diagnostic feature in the identification of the families Cupressaceae and Taxodiaceae.

Baker and Smith (1910) also commented on the structure of barks of the main Australian Coniferae and it is surprising that the modern bark authors referred to in this paper have not made reference to their work.

The structure of the phloem fibres is interesting in several ways. Firstly, the pits are numerous and definitely bordered, even though the pit chamber is quite small and sometimes indistinct. This is at variance with Chang's (1952) observation of some of the American Cupressaceae in which simple pits are reported. The combination of numerous pits and bordered pits in these phloem fibres is similar to the combination of numerous pits and bordered pits in fibre tracheids of xylem (I.A.W.A. Glossary, 1957). The pits which are aligned diagonally or radially are naturally blind, as the cells with which they make contact (sieve cells) have only primary walls through which conduction can occur. These blind pits of the fibres may coincide with sieve areas of the sieve cells. Pit pairs, naturally, are only found on the radial walls of the fibres.

No reference has been found in the literature as to whether the corrugated inner surface of the fibre wall occurs in other fibres of either phloem or xylem. Uneven inner cell walls resulting from thickening of pitted walls is, of course, well known in end walls of ray parenchyma cells in the xylem of the family Pinaceae.

The non-collapse of sieve cells of *C. macleayana* is also worthy of note, as collapse of sieve tubes is thought to be a characteristic of the older phloem (Holdheide, 1953). In other respects the sieve cells are the same as those described by Chang (1952).

While all the five species of *Callitris* examined have similar structures in their phloem (except in regard to the size and frequency of resin canals and length of fibres), the gross characteristics differ. These differences are due to the expansion of the parenchyma in the outer phloem and rhytidome. The large amount of expansion of phloem parenchyma in *C. hugelii*, *C. endlicheri* and *C. rhomboidea* disrupts the concentric fibre layers and the deposition of large amounts of "tannins" renders the mass of expanded cells compact. In *C. macleayana* the expansion of parenchyma is limited to occasional concentric layers allowing the fibrous nature of the phloem to be retained in the outer bark and permitting loosening of the fibrous layers. This loosening is assisted by the relatively small amount of tannin-like material deposited. *C. intratropica* retains the characteristics of the phloem in the rhytidome as only moderate expansion of parenchyma occurs. Chattaway (1954) has indicated the presence of expanded phloem parenchyma cells in certain species of *Eucalyptus* classified as "stringybarks". It is also suggested by Chattaway (1954) that this expansion of phloem parenchyma is associated with the death of these cells.

The occurrence of crystal sand in the middle lamella of the radial cell walls in certain species of *Callitris* and *Cupressus* appears of some interest, firstly, as crystalline deposits have rarely been recorded in the phloem cell wall (Chang records crystalline material in walls of *Taxus brevifolia* Nutt.), and secondly, as the material is laid down in continuous radial rows.

Baker and Smith (1910) concluded that the resin canals of *Callitris* were of lysigenous origin and that the resin (sandarac) was contained in cells or cavities. They attribute to this the difficulty of tapping *Callitris* trees so as to obtain a flow of resin as is done with turpentine. This, however, is not correct; the resin canals are

of schizogenous formation and the resin is secreted by the epithelial cells of the canal. The difficulty of obtaining a flow of resin is no doubt due to some physical characteristic of the resin as the canals appear to run vertically up the tree.

COMPARISON OF CALLITRIS AND CUPRESSUS BARK.

Bark of *Cupressus macrocarpa* grown in Sydney gardens (Pl. xxi, fig. 7) differs from *Callitris* in the following respects: (1) The concentric fibre layers are laid down irregularly, sometimes only four fibre layers being present in the entire phloem, and the bark is therefore softer than *Callitris*. (2) Resin canals are scarce in young trees sampled from Sydney. Although one sample from a large tree in Melbourne had a moderate number, the frequency of resin canals does not approach their high frequency in *Callitris*. (3) Tannin-like deposits of the phloem parenchyma are not dense.

It is suggested by Hadlington and Gardner (1959) that these marked differences in the structure of *Callitris* and *Cupressus* bark may account in some part for the differences in their susceptibility to attack by the cypress pine jewel beetle.

Examination of *Cupressus lusitanica* showed a moderate number of resin canals which agreed with Chang's (1952) comments for *C. lusitanica*. As in *C. macrocarpa*, the concentric fibre layers of *C. lusitanica* are irregular and the tannin-like deposits light.

IDENTIFICATION OF CALLITRIS SPECIES ON THE BASIS OF BARK ANATOMY.

The anatomy of the bark on the genus *Callitris* fits the key outlined by Chang (1952) for the classification of the North American Cupressaceae. Separation from the other genera of this family described by Chang (1952) can be made by use of the following features: (1) greater frequency of resin canals; (2) deposition of sandarac resin (Baker & Smith, 1910); (3) expansion of parenchyma cells in outer phloem and/or rhytidome.

A key for the identification of the five species examined here is suggested as follows:

1. No expansion of parenchyma in phloem. Expansion of occasional parenchyma layers in rhytidome *C. macleayana*.
- 1.* Parenchyma expanded in outer phloem 2.
2. Expansion of parenchyma moderate in outer phloem and rhytidome. Fibre layers regular in rhytidome *C. intratropica*.
- 2.* Expansion of parenchyma extreme in outer phloem and rhytidome. Fibre layers of rhytidome broken by parenchyma expansion 3.
3. Resin canals few, approximately 1-3 per sq. mm. *C. rhomboidea*.
- Resin canals from 6 to 13 per sq. mm. *C. hugelii* (Pl. xxi, fig. 8).
- Resin canals from 14 to 20 per sq. mm. *C. endlicheri* (Pl. xxi, fig. 9).

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EXPLANATION OF PLATES XX-XXI.

Plate xx.

1: Macerated phloem fibre of *Callitris endlicheri*. Corrugated inner surface of fibre and high frequency of pits are shown. Magnification $\times 600$. 2: Transverse section of *Callitris macleayana* phloem. A bordered pit is shown in a phloem fibre. $\times 540$. 3: Transverse section of *Callitris macleayana* bark. Periderm pe., phloem ph., rhytidome rh., expanded parenchyma ep. $\times 60$. 4: Transverse section of *Callitris endlicheri* bark. Expansion of phloem parenchyma in the outer phloem is shown. $\times 150$.

Plate xxi.

5: Transverse section of *Callitris intratropica* rhytidome. The slightly expanded parenchyma cells show abundant deposits of tanniniferous material. $\times 60$. 6: Transverse section of *Callitris rhomboidea* phloem. Expansion of phloem parenchyma and subsequent loss of concentric pattern is shown. The cambium side is at the bottom of figure. $\times 60$. 7: Transverse section of inner phloem of *Cupressus macrocarpa*. Three fibre layers can be seen crossing the section. $\times 48$. 8: Transverse section of *Callitris hugelii* phloem. Compare frequency of resin canals with Figure 9. $\times 60$. 9: Transverse section of *Callitris endlicheri* phloem. $\times 60$. 10: Radial longitudinal section of *C. endlicheri* phloem. Short swollen parenchyma cells (p.c.) occur between the long, thin phloem fibres (p.f.) and sieve cells (s.c.). The circular sieve areas (s.a.) of the sieve cells can just be seen. $\times 190$.

